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# Analysis of cross-reactivity of five new chicken monoclonal antibodies which recognize the apical complex of *Eimeria* using confocal laser immunofluorescence assay

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## Abstract

For Apicomplexa (members) the host cell invasion is realized with the help of the organelles located at the apical tip of parasites. In this research paper the characterization of five chicken monoclonal antibodies (mabs) produced against *Eimeria acervulina* sporozoites is described. All mabs reacted with molecules belonging to the apical complex of chicken *Eimeria* sporozoites. On immunofluorescence assay (IFA) one mab, 8E-1, recognized an apical tip molecule present on all chicken *Eimeria* sporozoites, two mabs (8D-2 and HE-4) recognized an antigen present on the apical tip of the same two *Eimeria* species (*E. acervulina* and *E. brunetti*), another mab (5D-11) recognized an antigen present on the apical tip of other two species (*E. acervulina* and *E. maxima*) while one mab (8C-3) identified antigens present on the sporozoites and sporocysts wall of only *E. acervulina*. Besides the apical tip antigens, two mabs (HE-4 and 8D-2) recognized some proteins located in the anterior half of the sporozoites. Collectively, these mabs proved that the apical complex of chicken *Eimeria* sporozoites share one or more antigens that are expected to play a role in host cell recognition and invasion.



*Keywords: Eimeria*; Apical complex; Chicken monoclonal antibodies; Immunofluorescence assay

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## 1. Introduction

Avian coccidiosis is an economically important disease for poultry industry worldwide. In chickens, seven distinct species of *Eimeria* which show predilection sites for invasion and intracellular development exist (Fernando, 1990). *Eimeria* has complex life cycle which includes asexual and sexual multiplication with stages that develop inside and outside the hosts. The worldwide rapid emergence of drug resistance (Chapman, 1993), the high costs associated with the development of new drugs and the public's concern over the drug-treated meat products (Cox, 1998) have recently prompted the development of alternative control strategies.

Although live and attenuated parasite vaccines have been widely used, live vaccines bear many disadvantages (reviewed in Vermeulen, 1998; Lillehoj and Lillehoj, 2000). Therefore, recombinant coccidia vaccines for coccidiosis control represent a strong candidate as an alternative control strategy. Host immunity is *Eimeria* specie-specific (Rose and Wakelin, 1990) and an effective recombinant vaccine, in the absence of identifying common protective antigens, should include antigens from several species and developmental stages of *Eimeria* (Jenkins and Dame, 1987; Jenkins, 1998). An ideal coccidia vaccine would include many antigens associated with host cell invasion and stimulation of protective cell-mediated immunity (Castle et al., 1991; Vermeulen, 1998; Lillehoj and Lillehoj, 2000).

Numerous mouse monoclonal antibodies which identify various antigens of *Eimeria* developmental stages, e.g., sporozoites, merozoites, gamonts, and oocysts have been described. However, when used as vaccines, the antigens identified using some of these mabs conferred only partial protection (Kim et al., 1989; Miller et al., 1989; Lillehoj et al., 1990; Jenkins et al., 1991; Bhogal et al., 1992; Vermeulen, 1998). The mechanisms that govern the antibody diversity in chickens are different from those in mammals (Reynaud et al., 1994) and indeed it has been shown that the immune sera from chickens, mice and rabbits react with different intensities with *Eimeria acervulina* sporozoites and merozoites antigens (Jenkins and Dame, 1987). For this reason, many studies were carried out to develop chicken hybridomas whose mabs can better identify coccidia antigens relevant to the natural host (Lillehoj et al., 1994; Sasai et al., 1996).

In previous studies, we showed that a chicken mab produced against *E. acervulina* sporozoites identified a common antigen present on the apical complex of the invasive stages of all chicken *Eimeria* and also on related coccidian parasites, *Toxoplasma* and *Neospora* (Sasai et al., 1996, 1998). The chicken mab designated 6D-12-G10 which shows a strong binding activity to the apical complex of all *Eimeria* species, significantly inhibited the sporozoites invasion of chicken $CD8^+$ lymphocytes (Sasai et al., 1996, 1998). Because of this initial success in identifying potential vaccine antigens of *Eimeria* using chicken mabs, we have now developed several additional chicken mabs which we characterize in this study using confocal immunofluorescence assay (IFA).

## 2. Materials and methods

Five chicken hybridomas were cloned, and their mabs designated as 8C-3, 5D-11, 8D-2, 8E-1 and HE-4 were characterized by indirect immunofluorescence and confocal laser

IFAs. These mabs were produced as described (Sasai et al., 1996) and all were of the immunoglobulin G class.

Seven species of chicken *Eimeria* were used in this study: *E. acervulina* Beltsville strain from US Department of Agriculture (USDA-USA), and *E. tenella*, *E. maxima*, *E. necatrix*, *E. praecox*, *E. mitis* and *E. brunetti* (National Institute of Animal Health, Tukuba, Japan). These parasites were passed in 3-week-old normal chickens and the oocysts preserved in 2% sodium dichromate at 4 °C.

Sporulated oocysts of *Eimeria* were obtained as described (Ryley et al., 1976). Oocysts were cleaned by hypochlorite treatment, their walls broken with 1 mm glass beads and the resulting sporocysts were incubated in excystation medium, 0.25% (w/v) trypsin (Merck, Darmstadt, Germany) and 1% (w/v) taurodeoxycholic acid (Sigma, St. Louis, USA) in Hanks' balanced salt solution (HBSS, Sigma, St. Louis, USA), pH 7.4 at 41 °C in a 5% $CO_2$ incubator. The released sporozoites were purified on diethylaminoethyl (DE52, Whatman International Ltd., Maidstone, Kent, UK) cellulose columns using a modification of the method described by Schmatz (Shirley, 1995).

The parasites in PBS were air dried on siliconized glass slides (Dako, Kyoto, Japan) at room temperature and fixed in cold methanol (2 min) followed by three washes with PBS. The parasites on the slides were incubated with 100 μl of each chicken mab for 30 min at room temperature or overnight at 4 °C in a humid box. Controls were incubated with tissue culture medium and chicken IgG (Cappel, ICN, Ohio, USA) resuspended in tissue culture medium. The slides were washed three times with PBS and then incubated with 100 μl of fluorescein isothiocyanate (FITC) conjugated rabbit anti-chicken IgG (1:1000, Sigma, St. Louis, USA) for 30 min and 12 h, respectively. The slides were washed three times with PBS and mounted with buffered glycerol. The parasites were visualized and photographed using either an Olympus confocal laser scanning microscope (Olympus, Tokyo, Japan) for *E. acervulina* or a Leica confocal laser scanning microscope equipped with an OG 515 filter (Leica AG, Heerbrugg, Switzerland) for all the other *Eimeria* species.

## 3. Results and discussion

For all Apicomplexa parasites, the apical complex which includes the apical tip, the conoid, polar rings and the secretory organelles (microneme, rhoptries and dense granules) is critically involved in host cell invasion. With the advent of chicken hybridoma technology, we have recently produced a stable mab (Sasai et al., 1996) that recognizes a conoid antigen shared among all chicken *Eimeria* sporozoites, *E. acervulina* merozoites, *T. gondii* and *N. caninum* tachyzoites. The immunization technique used in developing this mab allowed the identification of potential receptor molecules involved in host cell invasion.

In this study, we described the production of five new chicken mabs which detect *E. acervulina* sporozoites and further characterized their reactivity against other *Eimeria* species. The summary of the staining patterns of these mabs is shown in Table 1.

The mab 8C-3 stained a small region of the apical tip of only *E. acervulina* sporozoites and the sporocysts wall (Fig. 1A). This finding indicates that the mab 8C-3, similar to the mouse mab 15.84 described by Speer et al. (1989), recognizes a common protein shared among sporozoites and sporocysts of *E. acervulina*. The mab 5D-11 showed a weak staining of the

Table 1
Results of the confocal laser microscopic examination of chicken *Eimeria* sporozoites probed with the chicken monoclonal antibodies

| Species | mab 8C-3 | mab 5D-11 | mab 8D-2 | mab HE-4 | mab 8E-1 |
|---|---|---|---|---|---|
| *E. acervulina* | + | + | + | + | + |
| *E. brunetti* | | | + | + | + |
| *E. maxima* | | + | | | + |
| *E. mitis* | | | | | + |
| *E. necatrix* | | | | | + |
| *E. praecox* | | | | | + |
| *E. tenella* | | | | | + |

apical tip but a more intense staining at the middle portion of *E. acervulina* sporozoites. This mab also stained the apical tip of *E. maxima* sporozoites (Fig. 1B).

The mab 8D-2 stained the apical tip and the anterior half of *E. acervulina* sporozoites (Fig. 1C) and the apical tip of *E. brunetti* sporozoites. The mab HE-4 stained the apical tip and the anterior half of *E. acervulina* sporozoites (Fig. 1D) and the apical tip of *E. brunetti* sporozoites. The mab 8E-1 stained the apical tip of sporozoites of all chicken *Eimeria* (Fig. 2). This fact indicates that the mab 8E-1, like the 6D-12-G10 mab which we

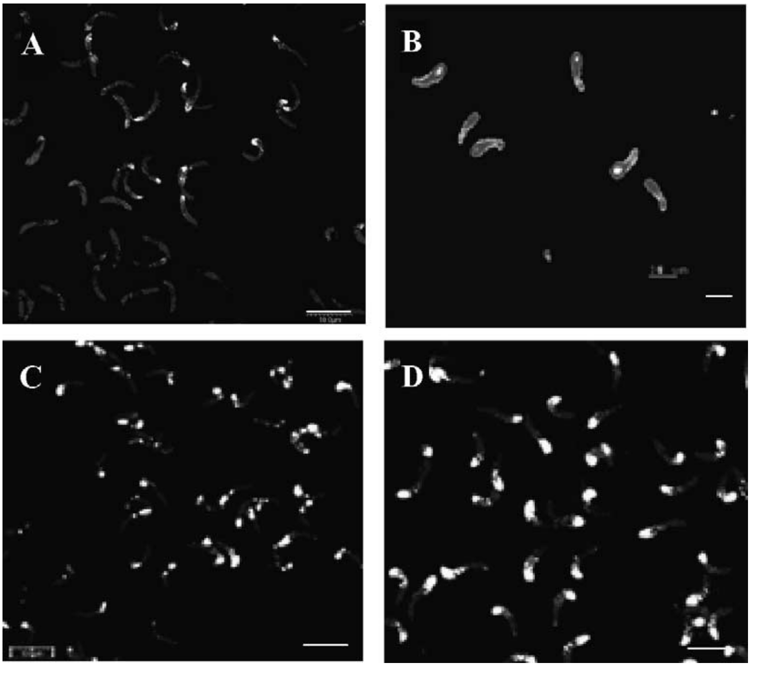


Fig. 1. Analysis of cross-reactivity of mabs 8C-3, 5D-11, 8D-2 and HE-4 using confocal laser microscopy: (A) *E. acervulina* sporozoites probed with mab 8C-3; (B) *E. maxima* sporozoites probed with mab 5D-11; (C) *E. acervulina* sporozoites probed with mab 8D-2; (D) *E. acervulina* sporozoites probed with mab HE-4. Bar in each picture represents 10 μm. Photos (A), (C) and (D) were taken with Olympus confocal laser scanning microscope and photo (B) with Leika confocal laser scanning microscope.

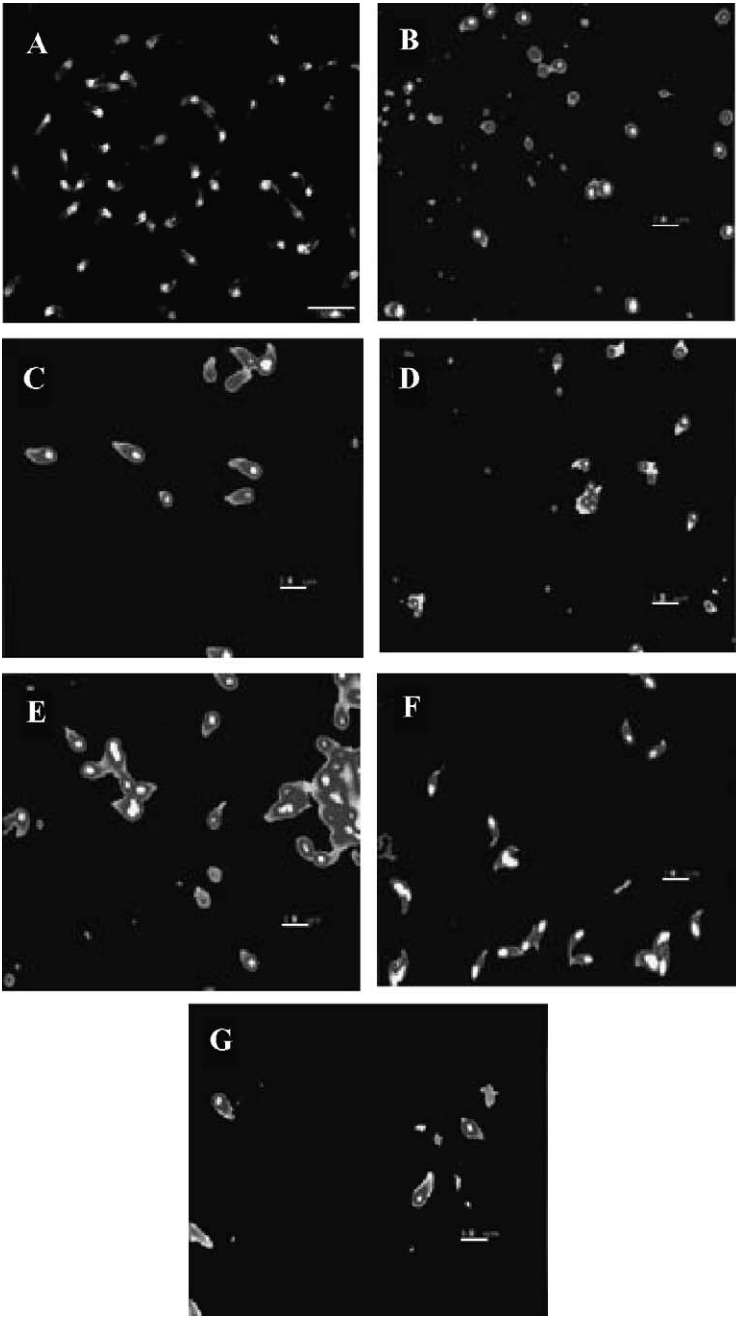


Fig. 2. Analysis of cross-reactivity of mab 8E-1 using confocal laser microscopy: (A) *E. acervulina* sporozoites; (B) *E. brunetti* sporozoites; (C) *E. maxima* sporozoites; (D) *E. mitis* sporozoites; (E) *E. necatrix* sporozoites; (F) *E. praecox* sporozoites; (G) *E. tenella* sporozoites. Bar in each picture represents 10 μm. Photo (A) was taken with Olympus confocal laser scanning microscope and photos (B)–(G) with Leika confocal laser scanning microscope.

previously described (Sasai et al., 1996), recognizes a cross-reactive epitope which might be important for the invasive stages of chicken *Eimeria*.

None of the chicken mabs recognized any proteins on Western blots, presumably due to the alteration of the epitopes identified by these mabs during electrophoresis or electrotransfer, a similar phenomena being encountered by other researchers using different mabs (Augustine et al., 1988; Lillehoj et al., 1994). Additionally, we were unable to localize the molecules on sporozoites using immuno-electron microscopy, presumably due to the changes that occurred during the specimen fixation or masked target antigens.

In summary, these new chicken mabs showed that there are apical antigens shared by two or all chicken *Eimeria* sporozoites or by sporozoites and other stages (sporocysts). The set of chicken mabs, including here the mab 6D-12-G10 (Sasai et al., 1996), developed by this laboratory showed that the chicken immune system recognizes many *E. acervulina* sporozoites apical antigens, of which four are shared with *E. brunetti*, three with *E. maxima* and two with all the other chicken *Eimeria*. The significance of this fact is unknown, somehow opposite to the results of Sutton et al. (1989) that examining the genetic similarities among chicken *Eimeria* concluded that *E. acervulina* is genetically closer to *E. tenella* than to the other *Eimeria*. The explanation could reside in the fact that not all polypeptides that make up an *Eimeria* species are antigenic and recognized by the host immune system. These chicken mabs, together with their mice counterparts, provide valuable tools for further characterization of *Eimeria* immunogenic antigens and will allow deciphering the intricate mechanisms of host recognition and invasion.

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